Replace the paragraph beginning at page 3, line 8 with:

D2 Based on the above patents a propofol preparation for clinical use (PDR 1996) has been commercially available (DIPRIVAN 1% Injection) which contains propofol dissolved in soybean oil and is stabilized with egg lecithin. Each milliliter of this formulation consists of 10 mg/mL of propofol, 100 mg/mL of soybean oil, 22.5 mg/mL of glycerol, 12mg/mL of egg lecithin, sodium hydroxide to adjust pH within 7 to 8.5 and sufficient quantity of water. Although clinically useful, this formulation requires the use of strict aseptic techniques during its handling due to the absence of antimicrobial preservatives and concomitant potential of microorganism growth. Indeed, many incidences of serious infection in human subjects have been linked to the use of the commercially available propofol formulation, DIPRIVAN (Nichols *et al.* (1995), Tessler *et al.* (1992), Ardulno *et al.* (1991), Sosis and Braverman (1993), Sosis *et al.* (1995), Crowther *et al.* (1996)).

Replace the paragraph beginning at page 3, line 20 with:

D3 In order to minimize the chances of infection arising from the handling of the formulations of propofol during intravenous administration Jones and Platt have recently introduced a new propofol formulation, essentially based on the earlier composition with the added component of an antimicrobial preservative. This product is described by US patents 5,714,520; 5,731,355; and 5,731,356. The antimicrobial preservative that is added to the new formulation is disodium edetate. In US patent 5,714,520 it is claimed that addition of an amount of edetate limits bacterial growth to no more than a 10-fold increase as determined by the growth of each of *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Candida albicans* ATCC 10231 for at least 24 hours as measured by a test wherein a washed suspension of each said organism is added to a separate aliquot of said composition at approximately 50 colony forming units (CFU) per mL, at a temperature in the range 20-25°C, whereafter said aliquots are incubated at 20-25°C and are tested for viable counts of said organism after 24 hours, said amount of edetate being no more than 0.1% by weight of said composition.

Replace the paragraph beginning at page 4, line 11 with:

However, regardless of the presence of edetate as a preservative against growth of microorganisms, the product under US patent 5,714,520 (DIPRIVAN) is not considered an antimicrobially preserved product under USP standards by some authors, for instance, Sklar (1997). While in the quantity that is present, edetate may be effective against the growth of some types of organisms that are claimed in the patent, it may not be so effective against a variety of other organisms that may be prevalent in the clinical situations where propofol is administered such as for example, *C. albicans* ATCC 10231 as noted in patent 5,714,520. Indeed, it was noted in patent 5,714,520 that the formulated propofol was not microbicidal against *C. albicans* ATCC 10231 where an approximately 10-fold growth in the inoculum concentration was observed after 48 hours. This result points to the possibility of ineffectiveness of edetate as a preservative against growth of microorganisms in Diprivan® formulation if challenged by other organisms than those cited above or by a higher load of organisms exceeding 100 CFU/mL. Indeed the addition of edetate to the formulation provides little in the way of real improvement. This "improved" formulation continues to be inferior, with respect to antibacterial effectiveness, to the invention described in the Haynes patent (US 5,637,625, see below).

Replace the paragraph beginning at page 5, line 8 with:

Many authors have reviewed the clinical usage of propofol formulations. For instance, Smith *et al.* (1994) describe that propofol injection has been used for producing and maintenance of ambulatory anesthesia, neurosurgical and pediatric anesthesia, for monitored anesthesia care, for intensive care sedation, and other clinical situations. Pain after injection of commercial formulations of propofol has been reported to occur in 28-90% of patients e.g., see reports by Mirakhur (1988), Stark *et al.* (1985), Mangar and Holak (1992). Even with low dose propofol administered for sedation, the incidence of pain can be 33-50%. (White and Negus, 1991; Ghouri *et al.* 1994). The mechanism responsible for the venous pain upon propofol administration is unknown. The original excipient, CREMOPHOR EL, of the earlier propofol preparation was initially thought to be the causative agent. However, there was no measurable reduction in pain after the change from the CREMOPHOR EL based propofol formulation to the

marketed soybean oil and lecithin based formulation (*e.g.*, see Mirakhur (1988), Stark *et al.* (1985), Mangar and Holak (1992). White and Negus, 1991; Ghouri *et al.* 1994). It is believed that the pain is a function of the drug itself, rather than the formulation (Smith *et al.* (1994)).

Replace the paragraph beginning at page 6, line 15 with:

While pain on injection may or may not be related to the injection-site tissue-irritation or the thrombogenicity of the administered formulation, these adverse reactions are still prevalent and symptoms continue to be reported in the clinical use of propofol. For instance, in the case of DIPRIVAN, these symptoms span the range of thrombosis and phlebitis and include up to 17.6% incidences of burning/stinging or pain (PDR 1999, p. 3416).

Replace the paragraph beginning at page 7, line 4 with:

Alternative propofol formulations, that addressed some of the above-mentioned clinical problems associated with the commercial (DIPRIVAN) or experimental (*e.g.*, those described by Babl et al. 1995, and Doenicke *et al.* 1996 and 1997) propofol injectable products, have been taught by Haynes in US patent 5,637,625. For instance, Haynes has recognized two problems associated with the use of large quantities of vegetable oil in a commercial formulation consisting of 1% propofol and 10% soybean oil:

(1) hyperlipidemia in patients undergoing long-term sedation in the intensive care unit (ICU), and

(2) the risk of bacterial contamination secondary to the high lipid content and lack of antimicrobial preservatives.

Replace the paragraph beginning at page 10, line 7 with:

At the surface of the water-insoluble matrix are amphiphilic agents that stabilize the dispersion and are of possible importance in affecting the degree of local reaction on injection. Examples of such amphiphilic agents include charged or uncharged phospholipids of natural sources, *e.g.*, egg or soy lecithin, or hydrogenated lecithin (*e.g.*, PHOSPHOLIPON-

90H™ or PHOSPHOLIPON-100H™ from Nattermann), or synthetic phospholipids such as phosphatidylcholines or phosphatidylglycerols, pharmaceutically acceptable non-ionic surfactants such as poloxamers (PLURONIC series of surfactants), poloxamines (TETRONIC series of surfactants), polyoxyethylene sorbitan esters (*e.g.*, TWEEN™ series of surfactants), cholesterol, or other surface modifiers commonly used in pharmaceutical products, or combinations of these surface modifiers.

Replace the paragraph beginning at page 11, line 15 with:

Propofol is a liquid that is very poorly soluble in water. To manufacture stable injectable propofol formulations with the desired anti-microbial properties, low lipid content and low injection site reactivity and with little or no phase separation of the propofol during mixing or storage, it was found necessary to not only select an appropriate composition of the formulation but also use appropriate processing conditions. Examples of suitable processing conditions are those which provide intense mechanical agitation or high sheer, see for example the procedures described by Haynes (US patent 5,637,625). The formulation is conveniently prepared by the initial preparation of a lipophilic phase and an aqueous phase which are then mixed. However, those skilled in the art will appreciate that alternate approaches may be suitable and will readily be able to determine such approaches. For example, the unit processes as described briefly in the following paragraphs have proven suitable.

Replace the paragraph beginning at page 14, line 2 with:

The circumference of the rat's tail was measured at approximately 2.5 inches proximal to the animal's body prior to the administration of the test formulation. This measurement served as a baseline value for assessing possible swelling of the tail upon intravenous administration of the formulation. On each study day, the treatment site was carefully examined to detect any reactions and the rat's tail circumference measured. Changes in the rat's tail circumference were evaluated by comparing Day 2 and Day 3 measurement to the baseline value obtained before administering the test articles.

Replace the paragraph beginning at page 15, line 17 with:

D12

The formulations described in the present inventions were tested for their ability to inhibit the growth of microorganisms that are a potential source of most likely infections in the clinical situation. Growth of *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 8739 and ATCC 8454), *Pseudomonas aeruginosa* (ATCC 9027), *Candida albicans* (ATCC 10231), and *Aspergillus niger* (ATCC 16403) was measured by a test wherein a washed suspension of each said organism is added to a separate aliquot of a formulation at approximately 1000 colony forming units (CFU) per mL, at a temperature in the range 20-25°C. The inoculated mixtures are incubated at 20-25°C. The viability of the microorganisms in the inoculated formulation is determined by counting the colonies of said organism after 24 and 48 hours, 7 days and other suitable lengths of time.

Replace the paragraph beginning at page 16, line 7 with:

D13

Unless otherwise specified, all parts and percentages reported herein are weight per unit weight (w/w), in which the weight in the denominator represents the total weight of the formulation. Diameters of dimensions are given in millimeters (mm = 10^{-3} meters), micrometers (μm = 10^{-6} meters), or nanometers (nm = 10^{-9} meters). Volumes are given in liters (L), milliliters (mL = 10^{-3} L), and microliters (μL = 10^{-6}L). Dilutions are by volume. All temperatures are reported in degrees Celsius. The compositions of the invention can comprise, consist essentially of, or consist of the materials set forth and the process or method can comprise, consist essentially of, or consist of the steps set forth with such materials.

Replace the table beginning at page 17, line 1 with:

Raw Material	Symbol	Source
1,2-Dimyristoyl-sn-Glycero-3-Phosphocholine	DMPC	Avanti Polar Lipids Inc., Alabaster, AL, US
1,2-Dimyristoyl-sn-Glycero-3-[Phospho-rac-(1-glycerol)]	DMPG	Avanti Polar Lipids Inc., Alabaster, AL, US
Ethyl Oleate, NF	EO	Croda Leek Ltd, Staffordshire, UK
Glycerin, USP-FCC	GLY	J.T. Baker, Philipsburg, NJ, US
LIPOID E80™ (egg lecithin)	E80	Lipoid GmBH, Ludwigshafen
LIPOID EPC™ (egg phosphatidylcholine)	EPC	Lipoid GmBH, Ludwigshafen
LIPOID SPC™ (soy phosphatidylcholine)	SPC	Lipoid GmBH, Ludwigshafen
LIPOID SPC-3™ (saturated soy phosphatidylcholine)	SSPC	Lipoid GmBH, Ludwigshafen
Mannitol, USP	MAN	J.T. Baker, Philipsburg, NJ, US
MIGLYOL 810™	M810	Hüls America, Piscatway, NJ, US
Propofol	PRO	Albemarle Corporation, Baton Rouge, LA, US
Soybean oil, USP	SO	Spectrum, New Brunswick, NJ, US
(D+) Alpha, alpha-Trehalose	TRE	Pfanstiehl Laboratories Inc, Waukegan, IL, US

Replace the paragraph beginning at page 17, line 4 with:

Table I summarizes some examples of the propofol formulations and their attributes with increasing amount of oil. The oil concentration of these formulations was increased by increasing the amount of ethyl oleate from 0.4% to 10%. Propofol concentration was kept at 1%. Amount of the phospholipid mixture (LIPOID E80 and DMPG) was adjusted with increasing amount of oil to obtain the formulations of good stability.

Replace the paragraph beginning at page 18, line 4 with:

D16

Rat-tail swelling, an indicator of the tissue-irritation propensity of the formulation (see above), was found to decrease with increasing amount of oil. Formulation #1.4-1.6 with 4-10% ethyl oleate appear to result in unnoticeable rat-tail swelling. This result parallels the reported finding (Babl et al. 1995, and Doenicke *et al.* 1996, and 1997) that the use of higher amounts of oil in propofol preparations reduces the incidence of pain on injection possibly by a reduction of aqueous concentration of propofol. However, these authors have used a much higher amount (20%) of MCT and LCT mixture in their propofol formulations, and such formulations are expected to support the growth of microorganisms.

Replace Table I beginning on page 18, line 13 with:

Table I: Effect of increasing oil content of the formulation

D17

Formula-tion ID	Propofol (%, w/w)	LIPOID E80 (%, w/w)	DMPG (%, w/w)	Ethyl Oleate (%, w/w)	Viscosity, cP	Rat Tail Swelling, at 48hr, mm	LDH (IU/L)
1.1	1	0.8	0.15	0.4	0.97	1.39	10918
1.2	1	0.8	0.10	1.0	1.08	0.6	10970
1.3	1	0.8	0.10	2.0	1.06	0.2	10300
1.4	1	1.0	0.25	4.0	1.04	0	3150
1.5	1	1.0	0.25	8.0	1.25	0	1290
1.6	1	1.0	0.25	10.0	1.34	0	770

Replace the paragraph beginning at page 20, line 11 with:

D18

In Example 1 it was observed that by increasing the amount of oil from 0.4% to 10% or greater in the formulation, the tissue-irritation potential could be decreased. However, Example 2 indicates that this simplistic notion is not without limitation since in some cases merely increasing the amount of oil in the propofol formulation does not result in a less irritating formula. For instance, in formulation 2.26 the oil level is increased to 6% of ethyl oleate and in formulations 2.27 and 2.28 to 4% of MIGLYOL 810, but these formulations are still injection-site tissue-irritating, which is evident from the tail swelling values for these formulations.

Replace the paragraph beginning at page 21, line 11 with:

D19

As established in Example 1 and again here in Example 2, merely increasing the oil level in formulations did not result in decreasing the hemolytic potential, or irritation to the tissues at the site of injection. It appears that below a certain amount of oil (*e.g.*, <10%) the causative factors for improving the hemolytic potential or tissue irritation is a combination of various factors that originate from the specific composition. Thus, the non-irritating formulations that also have a low potential of hemolysis are characterized by various formulation components that provide the co-operative effects rendering the preferred formulations less irritating.

Replace the paragraph beginning at page 21, line 20 with:

D20

Whether the formulations demonstrated the absence of thrombogenic irritation in rats or caused such irritation, all were examined for the microbicidal or microbistatic effectiveness as mentioned above of which some relevant results are summarized in Table III. Also presented in Table III are the microbicidal effectiveness test results for DIPRIVAN as a comparison.

Replace the footnote "Symbols and Note" at page 24, after Table II: Continued with:

D21

DMPC: dimyristoylphosphatidylcholine; DMPG: dimyristoylphosphatidylglycerol; E80: LIPOID E80; EO: ethyl oleate; EPC: egg phosphatidylcholine; EPL: egg phospholipids; GLY: Glycerin; M810: MIGLYOL 810; MAN=Mannitol; SO: soybean oil; SPC: soy phosphatidylcholine; SSPC: saturated soy phosphatidylcholine; TRE=Trehalose. Sources of these raw materials are mentioned above.

Replace Table III at page 25, line 1 with:

Table III: **Log growth of certain microorganisms following an initial inoculation of 10^3 CFU/mL in presence of some propofol formulations.**

Formulation ID of Example 2	Organism	*C. albicans* ATCC 10231			*P. aeruginosa* ATCC 9027			*E. coli* ATCC 8739			*A. niger* ATCC 16403			*S. aureus* ATCC 6538		
	Formulation / Plating Time	24 hr	48 hr	7 day	24 hr	48 hr	7 day	24 hr	48 hr	7 day	24 hr	48 hr	7 day	24 hr	48 hr	7 day
2.1	91.103	2.8	2.7	2.1	1.5	1.0	1.0	1.7	1.6	1.0	2.8	2.8	2.7	2.4	1.0	1.0
2.3	61.103	2.8	2.3	1.3	1.0	1.0	1.0	1.0	1.0	1.0	2.0	2.1	2.0	1.0	1.0	1.0
2.4	76.103	2.7	2.7	2.5	2.3	1.3	1.0	2.5	2.1	1.0	2.9	2.8	2.7	2.2	1.0	1.0
2.5	81.103	3.0	3.9	6.0	2.4	6.0	6.8	4.8	6.8	6.8	2.8	2.6	2.5	3.3	3.2	1.3
2.6	80.103	2.9	3.4	5.8	1.0	1.0	1.0	4.4	6.8	6.8	2.8	2.6	2.6	3.0	2.5	1.0
2.11	72.103	3.1	4.0	5.8	2.3	5.9	6.8	5.1	6.8	6.8	2.9	2.7	2.6	3.2	3.1	1.0
2.13	50.103-A	4.2	5.0	5.3	2.0	3.7	6.8	1.0	1.0	1.0	2.5	2.4	2.1	2.0	1.0	1.0
	DIPRIVAN	3.2	3.4	3.2	2.8	3.3	6.2	2.2	1.0	1.0	2.9	2.7	2.6	3.2	3.1	1.8

Replace the paragraph beginning at page 26, line 4 with:

As taught by Haynes (US patent 5,637,625) it may be thought that increasing the amount of lipidic nutrients in the formulation would cause the formulation to support microorganism growth. However, it is surprising to note that by increasing the amount of oil (to up to 4-6%), formulations 2.1, 2.3 or 2.4 do not provide a medium for bacterial growth. It is worth noting that formulations 2.1, 2.3, and 2.4 were neither irritating, nor hemolytic while also inhibiting the growth of microorganisms. These non-irritating, non-hemolytic, and bactericidal or bacteristatic formulations are characterized as non-limiting examples of preferred compositions of this invention.

Replace Table IV beginning at page 26, line 16 with:

D24

Table IV: Propofol formulations of high drug potency

	Formula 4.1	Formula 4.2	Formula 4.3
Propofol	5.0%	10.0%	10.0%
Cholesterol	0.25%	0.4%	0.5%
Cholesteryl oleate	---	4.0%	3.0%
PHOSPHOLIPON 90H	1.5%	1.8%	1.5%
DMPG	0.3%	0.3%	0.15%
Glycerol	2.5%	2.5%	2.5%
Sodium hydroxide	qs pH 6.9	qs pH 8.2	qs pH 7.0
Water	qs 100%	qs 100%	qs 100%

Replace the paragraph beginning at page 28, line 2 with:

D25

Propofol formulations of this invention were compared for induction and duration of anesthesia in rats with the reference commercial formulation, DIPRIVAN (1%) and DISOPRIVAN (2%). Following 12.5 mg/kg single bolus intravenous injection of these formulations in rats, the time for loss of consciousness and righting response time were measured as mentioned above in the experimental method section. The results are summarized in Table V illustrating the efficacious characteristic of these formulations.

Replace Table V beginning at page 28, line 8 with:

Table V: Pharmacodynamic Parameters

Formulation ID	Number of Rats	Average Anesthesia Induction Time (sec)	Average Righting Response Time (min)
2.1	9	24.4	14.9
2.2	4	31.0	16.2
2.3	4	48.0	16.4
2.4	9	32.7	15.8
2.5	9	27.2	19.2
2.6	9	38.4	19.4
2.7	4	24.0	17.3
2.8	4	23.8	16.7
2.9	3	67.3	11.9
2.10	4	34.8	16.3
2.11	8	40.5	18.6
2.12	4	36.3	13.7
DIPRIVAN (1% with EDTA)	4	20.0	14.6